

AMENDMENTS

In the Specification:

Page 1, please replace the paragraph beginning at line 14 with the following:

Gene products are prepared in large quantities in microorganisms using a variety of recombinant DNA techniques. Such techniques involve selection of an appropriate host, increasing the number of gene transcripts, improving translation efficiency, and improving the stability of the proteins themselves. To increase the number of gene transcripts ~~transcript~~ for high-level production of gene products, it is important to use both an effective transcription promoter and to maximize the number of copies of the gene-expression unit. Typically this comprises increasing the amount of transcription promoter/terminator sequences as well as the gene to be expressed. This increases transcript numbers ~~number~~ as a whole.

Page 1, please replace the paragraph beginning at line 22 with the following:

For industrial scale production, gene-expression units can be stably maintained in microbial cells. Plasmid vectors are at a disadvantage in this regard and generally stabilized by integration into a chromosome. It has been reported that dozens of copies of a vector could be integrated into the ribosomal RNA gene (rDNA) regions of a yeast cell by using the vector carrying a transformation marker gene in which the promoter region was truncated ~~truncated~~ to reduce expression level (Lopes T. S. et al., Gene, 79, 199-206, 1989; Bergkamp R. J. M. et al., Curr. Genet., 21, 365-370, 1992; Le Dall M. T. et al., Curr. Genet., 26, 38-44, 1994).

Page 2, please replace the paragraph beginning at line 16 with the following:

One embodiment of the invention is directed to vectors comprising 5S rDNA. These vectors contain a portion of the *S. cerevisiae* ~~cerevisia genome-genome~~, namely, a 2.1 kbp EcoRI-EcoRI fragment containing a 5S rDNA gene and spacer ~~spacer~~ regions NTS1 and NTS 2. Vectors may

also contain antibiotic resistance genes such as, but not ~~[[ot]]~~ limited to, genes for ampicillin resistance, tetracycline resistance, and G418 resistance.

Page 3, please replace the paragraph beginning at line 11 with the following:

5S rDNA vectors of the invention were used to transform various strains of yeast cells including wild-type yeast strains, strains of the genus *Saccharomyces*, and strains of other non-*Saccharomyces* genus (e.g. *Candida*). The basic 5S rDNA vector was formed from a 2.1kb EcoRI-EcoRI *S. cerevisiae* rDNA ~~fragments~~ fragment that includes the 5S gene and the NTS1 and NTS2 spacers from *S. cerevisiae*. In one embodiment, a ~~glycol-amylose~~ glycoamylase gene expression cassette of *Aspergillus awamory* is inserted into the HpaI site of the NTS1 spacer (p1-9g18 vector). In another embodiment, the geneticin (G418) resistance gene is inserted into the HpaI site of the NTS1 spacer (pA-4). In another embodiment, the geneticin (G418) resistance gene inserted in the HpaI site of the NTS1 spacer, and the ~~glycol-amylose~~ glycoamylase gene expression cassette of *Aspergillus* awamory ~~awmory~~ is inserted into the HindIII site of the NTS1 spacer (pGG7).

Page 6, please replace the paragraph beginning at line 29 with the following:

For dot blot experiments, total yeast DNA was denatured by incubation for 5 minutes at 100°C and 10 minutes on ice, and blotting on nylon membrane (Hybond N; Amersham Pharmacia Biotech). For Southern Blots, yeast chromosomes were separated in Contour-clamped Homogeneous Electric Fields- CHEF, in Chef Mapper and Chef Mapper XA Pulse Field Electrophoresis Systems (BioRad). ~~Chromosomes~~ Chromosome samples were prepared ~~prepare~~ according to the manufacturer's protocol. After electrophoresis, DNA was transferred to nylon membranes using a capillary protocol (Sambrook *et al.*, 1989). When ~~the vector was introduced to~~ *Aspergillus awamory*, glycoamylase expression cassette in the yeast chromosome, the probe was 2.1kb *SmaI-BglII* fragment of the glycoamylase structural gene. When vector carried only the G418

gene, the probe was the 1.28 kb *EcoRI*-*EcoRI* fragment from pUC4K plasmid. Hybridization experiments used AlkPhos Direct (Amersham Pharmacia Biotech).

Page 8, please replace the paragraph beginning at line 1 with the following:

The ~~fragment 2.1 KB *EcoRI*-*EcoRI* fragment of 2.1 KB~~ from the plasmid YIpRH with the 5S rDNA gene was inserted in the *EcoRI* site of YIp352ssh plasmid and referred to as p1-9. The glycoamylase expression cassette of *A. awamory*, present in the fragment *HindIII*-*HindIII* of plasmid YEp-EIJ², was inserted into the *HindIII* site of plasmid p1-9 forming p1-9g18. The 1.28 kb *EcoRI*-*EcoRI* fragment that includes ~~count~~ the gene G418 of plasmid pUC4K, previously treated ~~treaty~~ with the Klenow fragment of DNA polymerase (fragment *EcoRI*-*EcoRI*/Klenow), was inserted in the ~~site~~ *HpaI* site in plasmid p1-9, making the new plasmid ~~was~~ pA-4. The fragment *EcoRI*-*EcoRI*/Klenow of pUC4K, with the G418 gene, was inserted into the *HindIII* site of p1-9g18. The resulting plasmid was referred to as pGG7. The 5S rDNA vectors, named p1-9g18, pA-4 and pGG7 (Figure 2), were obtained after the treatment of each ~~one~~ of the new plasmids with the *EcoRI*. However, to facilitate the vector DNA preparation, each was subcloned into the *EcoRI* site of the pUC18 plasmid. Resulting plasmids were referred to as pUC1-9g18, pUCA-4, pUCGG7.